STUDIES ON THE GROWTH HORMONE OF PLANTS

VI. The Distribution of the Growth Substance in Plant Tissues

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Recent studies on the growth hormone of plants have indicated its very wide distribution in both the plant and animal worlds. Its function in promoting growth by cell elongation, while so far as we know confined to plant tissues, is, nevertheless, of a completely non-specific nature. Coleoptiles of Gramineae, stems of Commelinaceae, flower stalks of Compositae, stems of Leguminosae and the gynostemia of some orchids are all subject to the influence of the hormone, and, so far as cell elongation is concerned, are probably completely dependent upon it. Nevertheless, the presence and quantity of the growth hormone in many plant tissues is difficult to establish, largely because, although soluble in water, the substance appears not to be extracted on grinding the tissues with water. Thus, Went (1928) was able to extract growth substance from coleoptiles of *Avena* by placing them on agar but not by grinding with water. In the course of various attempts to determine the growth substance in plant tissues, it was found that in general only small amounts could be extracted on grinding with water, while extraction with organic solvents often yielded considerable amounts of the hormone.

Furthermore, it was shown in Part I of this series (Dolk and Thimann, 1932) that the growth hormone is a relatively strong acid, of pK = 4.75, and hence it follows that in order to extract it in organic solvents the aqueous tissue must be brought to a hydrogen ion concentration of at least 10^{-3}. It must also be remembered that the amount of growth hormone which will produce a curvature of 1° in a standard *Avena* coleoptile when present in an agar block of volume 10.7 mm.^{3} (= one plant unit) is only 1/200 of the amount which must be present in 1 cc. of liquid, so that in order to detect the minute amounts of growth

substance present in coleoptiles and similar tissues, the extracts must be made up to as small a final volume as possible.

These considerations led to the following technique for extraction: The fresh material is killed by immersing in chloroform, about one-fifth of its volume of 1 N HCl is added, and the mixture thoroughly ground. The chloroform layer is separated off, and the acidified tissue ground twice more with chloroform. Ether is not used on account of the destructive effect on growth substance of the traces of peroxide always present in ether (*cf.* Dolk and Thimann, 1932). Smaller amounts of peroxide may also be present in chloroform particularly after exposure to light, but these amounts are usually too small to produce any appreciable inactivation. Finally, the chloroform is evaporated off and the lipoidal material taken up in a very small volume of water, usually 0.3 cc., and tested by adding an equal volume of 3 per cent agar, pipetting 0.5 cc. of the mixture into

TABLE I

Comparison of Water and Chloroform Extractions

Material	Growth substance in plant units per gm. or piece		Ratio $\frac{\text{Growth substance in } CHCl_3}{\text{Growth substance in } H_2O}$
	$CHCl_3$ extraction	H_2O extraction	
Wheat germ	320,000 per gm.	31,000 per gm.	10
Pollen of *Sequoia*	4,000,000 per gm.	160,000 per gm.	25
Terminal buds of *Vicia faba*	46.1 per bud	9.6 per bud	5
Coleoptile tips of *Avena*	5.8 per tip	4.7 per tip	1.2
Coleoptile bases of *Avena*	6.9 per base	1.3 per base	5

the standard brass ring, and cutting into blocks of volume 10.7 mm.^{3} The amount of growth substance which must be in such a block to give a curvature of 1° is one plant unit, which is equal to 0.4 × the *Avena* unit of Kögl, Haagen-Smit, and Erxleben (1933).

Table I compares the amount of growth substance obtainable in this way from a few representative plant tissues with the amount set free by simple extraction with water. The water extractions were made by adding acid as before and grinding as thoroughly as possible three or four times with small volumes of water. It may be noted that by this method the pollen of *Sequoia* is seen to be an even better source than the pollen of orchids used by Laibach (1933). The growth substance obtainable from buds of *Vicia* is approximately

equal to the amount which would diffuse out into agar in 1 hour, as determined in a previous communication (Thimann and Skoog, 1933). This indicates that the growth substance would occupy 1 hour in travelling from the extreme tip of the bud to the cut surface. Since this distance is about 1 cm., the velocity of movement of the growth substance is 1 cm. per hour, a figure which agrees with the determinations of Van der Wey (11 mm. per hour in Experiment 80 and 10 mm. per hour in Experiment 79; 1932). The ratio in the last column

TABLE II

Inactivation of Growth Substance by Leaf Extract

Time of contact between growth substance and leaf extract about an hour.

Concentration of growth substance solution used	Activity in units per cc.		Ratio $\frac{A_{H_2O}}{A_{LE}}$	
	In water	In leaf extract		
(*a*) *Vicia faba.* Catechol oxidase present				
a	40.2°*	8.8°	4.5	4.4
a/2	20.1°*	4.7°	4.3	
(*b*) *Helianthus annuus.* Catechol oxidase present				
b	15.6°	4.7°	3.3	2.8
b	15.6°	4.4°	3.5	
b	15.6°	6.5°	2.4	
b/2	7.8°	3.7°	2.1	
(*c*) *Malva parviflora.* Catechol oxidase absent				
a/2	20.1°*	13.5°	1.5	1.3
a/4	10.0°	8.8°	1.1	

* Assay at higher dilution.

shows that, with the exception of coleoptile tips, the chloroform extraction yields at least five times as much growth substance as water extraction.

In order to prove that the low yields obtained on extraction with water are due to inactivation of the growth substance by plant enzymes the following experiment was carried out. A leaf extract was made by crushing 5 gm. of *Vicia faba* leaves in a little water and making up to 4 cc. A concentrated growth substance solution was then diluted

with this extract and with water in parallel experiments and the two solutions tested. The results, summarized in Table II (*a*), show that the leaf extract causes a great inactivation of the growth substance and that the ratio of activity in leaf extract to activity in water (last column of the table) is of the same order as that between chloroform and water extracts, as given in Table I. A leaf extract made from *Helianthus annuus* gave similar results (Table II (*b*)). It has also been reported, but without details, by Kisser *et al.* (1931) that the addition of crushed leaves to a growth substance solution resulted in inactivation.

Since it was shown in Part I of this series (Dolk and Thimann, 1932) that the activity of the growth substance was destroyed readily on oxidation, it seemed probable that the inactivation by leaf extract is due to oxidation by the peroxidase-catechol-oxidase system, which is widely distributed among plants. If this is so, then a leaf extract made from a plant which does not possess this system should not cause inactivation.[1] This was proved by an experiment, carried out exactly as above, but using an extract from the leaves of *Malva parviflora*. It was shown by Onslow (1921) that the Malvaceae are lacking in this enzyme system. The results, in Table II (*c*), show almost no inactivation by this extract. Hence, the inactivation is probably an oxidation, and since the majority of plants contain this or a related oxidase system, it follows that as a general procedure the grinding of plant tissues with water is undesirable, as it will lead to the oxidation of the growth substance contained in them. This fact provides an explanation for the results of Cholodny (1931), who showed that after wounding, the sensitivity of the wounded side of *Lupinus* hypocotyls to light and gravitation was greatly reduced. This phenomenon is thus to be ascribed to enzymic inactivation of growth substance and not necessarily to a special wound hormone.

The Distribution of Growth Substance in Avena Coleoptiles

Since the placing of coleoptiles on agar after they have been decapitated yields no growth substance, it has generally been assumed that

[1] The dihydroxyphenylalanine oxidase system of *Vicia faba* is to be regarded as a special form of the catechol oxidase.

the substance is used up in the growth reaction and disappears from the coleoptile. For studies on the mechanism of the action of the hormone, it is desirable to know whether it really disappears or not from the tissues in which it has reacted. The above simple method of extraction, while it cannot determine whether or not the substance is used up in the growth reaction itself, can at least determine whether growth substance is present in the lower parts of the coleoptiles and if so to what extent.

It has already been shown (Table I) that the substance is, in fact, present in detectable amounts in the bases of the coleoptiles. Table III summarizes a number of determinations made on tips and bases. In order to clarify the details of the method, all the measurements are given in full. The last column shows that the number of plant units per millimeter of coleoptile length is fairly constant, and the amount in the base is unexpectedly large, being about half that in the tip.

TABLE III

Growth Substance Extractions from Avena Coleoptiles

	No. taken	Length	Volume of final solution	Curvature	No. of test plants	Growth substance per piece in plant units	Growth substance per unit length in plant units per mm.	Average plant units per mm.
		mm.	*cc.*					
Tips	90	5	0.5	4.6°	11	5.2	1.04	
	128	5	0.4	9.3°	19	5.8	1.16	
	99	5	0.5	3.5°	17	3.5	0.70	
	166	5	0.6	19.6°*	35	7.2	1.45	
	89	5	0.6	4.6°		3.1	0.61	0.99
Bases	128	15 av.	0.4	11.1°*	21	6.9	0.46	
	166	22 av.	0.9	35.2°*	52	12.7	0.58	
	89	22 av.	0.6	7.3°	26	9.9	0.44	0.49

* The angles in these instances were determined by diluting the agar blocks according to the method of Went (1928).

A closer analysis of the distribution was made by dissecting a large number of coleoptiles of the same age, the primary leaf being removed; the sections extracted were as follows: (1) the topmost 2 mm.; (2) the

next 3 mm.; (3) the next 3 mm.; (4) the next 5 mm.; and (5) the remainder. The total length of these remainders was measured and the average obtained. The results of four such experiments are given in Table IV. While there is marked variation in the absolute amounts which is no doubt due to the extremely small quantities dealt with, the type of polar distribution is the same throughout. In Fig. 1 the

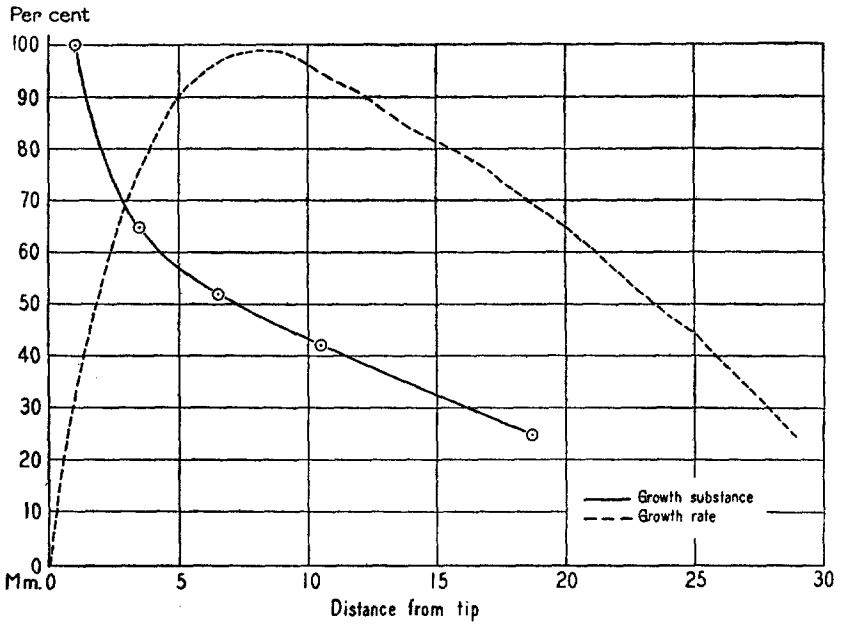

FIG. 1. Distribution of growth substance and growth rate in the *Avena* coleoptile. Solid curve, growth substance as per cent of the amount in the 2 mm. tip; mean of four experiments in Table IV. Broken curve, growth rate as per cent of the maximum rate; mean of four experiments by F. W. Went (three unpublished). Average length of coleoptiles extracted, 26 mm.

mean distribution, in per cent of the amount in the tip, is contrasted with the distribution of growth rates as given by Went. While the amount of growth substance per millimeter falls off steadily with distance from the tip, the rate of growth, as is well known, passes through a well defined maximum. There is no accumulation of growth substance in the middle of the coleoptile, and the view of Went that another factor limits growth in the apical portion is thus borne out.

The amount of growth substance diffusing out from tips when placed on agar was determined for comparison. It proved to be about five plant units per tip per hour, and to be maintained for at least 6 hours. There is therefore no doubt that the tip continues to produce growth substance when cut off, since by extraction only about 1 hour's supply can be obtained. From these experiments it follows that the growth substance does not immediately disappear from the tissues. On the other hand, since cut off sections of coleoptile continue to grow for some time, it cannot be argued that the growth substance which is extracted by chloroform has already taken part in the growth reaction. It was shown by Bonner (1934) that decapitated coleoptiles still con-

TABLE IV

Distribution of Growth Substance in the Coleoptile

Section No.	Length	Experiment 1		Experiment 2		Experiment 3		Experiment 4		Average as per cent of plant units per mm. in tip
		Plant units per section	Plant units per mm.	Plant units per section	Plant unit per mm.	Plant unit per section	Plant unit per mm.	Plant units per section	Plant unit per mm.	
	mm.									
I	2	4.91	2.46	1.66	0.83	1.12	0.56	1.33	0.67	100
II	3	—	—	1.40	0.47	1.35	0.45	1.33	0.44	65
III	3	5.20	1.73	1.04	0.35	1.22	0.41	0.92	0.37	52
IV	5	2.82	0.56	1.20	0.24	1.50	0.30	1.66	0.33	42
V	Base	4.70	0.34	1.34	0.11	2.87	0.37	1.13	0.09	25
Average length of coleoptile		27.0 mm.		25.6 mm.		22.2 mm.		25.8 mm.		

tain enough growth substance to give a curvature when acid is admitted to the tissues. It seems therefore that the growth substance is present in two different forms, one, as in tips of coleoptiles, in which it diffuses out into agar, and one in which it does not, although it is still extractable from the tissue. Tentatively it may be suggested that the first, or free, form is the one which is redistributed in the coleoptile under the influence of light or gravity and is thus responsible for tropisms. The growth substance which is set free from its salts by the action of acid, as in the experiments of Bonner, is principally in the second or bound form, since it does not diffuse out of the coleoptile, but is obtained by extraction.

Distribution of Growth Substance in Roots

Some conflict of opinion has arisen in regard to the presence of growth substance in roots. Thus Gorter (1932) was unable to confirm the work of Cholodny (1928) and others that decapitated roots will show a geotropic response if root or coleoptile tips be applied under the right conditions. While the conflict is largely resolved by the recent paper of Cholodny (1933), no conclusive evidence has been brought forward to show whether growth substance is produced in the root tip or not. However, Boysen-Jensen (1933), by using dextrose agar, has been

TABLE V

Distribution of Growth Substance in Avena Roots

Section	Total length of combined sections	Angle	No. of plants	Total No. of plant units	Plant units per mm.	Per cent
	mm.					
(1) 1st 10 mm.	1500	5.1°	19	·306	0.204	100
(2) Base	5250	12.7°	23	762	0.145	73
(1) 1st 10 mm.	1410	8.5°	18	510	0.362	100
(2) 2nd 10 mm.	1410	7.2°	17	432	0.306	84.5
(3) Base	3713	13.5° / 7.5 + 8.2°	11 / 10	876	0.235	65
(1) 1st 10 mm.	1240	14.7° / 2 × 8.6°	11 / 12	960	0.774	100
(2) 2nd 10 mm.	1250	13.1°	18	786	0.629	81
(3) Base	3550	2 × 11.5° / 4 × 5.9°	11 / 11	1400	0.394	51

able to cause growth substance to diffuse out from roots of *Zea*, and has shown that the amount so obtained decreases steadily with increasing distance from the tip. However, his experiments, while very valuable, still do not show whether it is the actual concentration of growth substance present, or only the ease with which it diffuses out, which decreases with distance from the tip. If it could be shown by direct extraction that the concentration really increases towards the tip this would go far towards settling the question. Such experiments were therefore undertaken.

The roots of *Avena*, grown in water in the usual way, were rubbed in running water to free them from adhering bacteria, etc., and dissected into three portions, the tip 10 mm., the second 10 mm., and the basal part, which varied in length from 10 to 40 mm. A large number of these were obtained at one time and extracted. The results, Table V, show not only that growth substance is present in roots, but also that its distribution is polar; *i.e.*, that its concentration is greatest at the tip. When it is further considered that the apical end of the root tapers somewhat, the amount of growth substance per unit of weight would show an even more marked polar distribution. The concentration of

TABLE VI

Growth Substance Diffusing out of 10 Mm. Root Tips into Dextrose Agar

No. of roots	Time on agar blocks	No. of blocks	Angle	Plant units per root	Control
	hrs.				
40	1.5	12	2.7°	0.8	+1.0°
20	3	6	4.0°	1.0	
20	12	6	2.3°	0.7	
24	18	12	5.1°	2.6	
20	23	12	5.3°	3.2	0.0°
36	24	6	16.5°	3.5	
21	46	12	6.4°	3.7	
36	48	12	11.1°	3.7	
30	72	12	6.5°	2.6	+1.1°
20	72	12	4.7°	2.8	−2.0°
By extraction; mean of three experiments in Table V.....				4.35	

growth substance per unit length is somewhat less in the root tip than in the coleoptile tip, but in the root base it is about the same as in the coleoptile base. The extent of the decrease with distance from the tip is comparable to that in the coleoptile, and would seem to indicate that growth substance is produced in the tip.

On the other hand, if growth substance is produced in the root tip then it should be possible by diffusion to obtain it in larger amounts than can be found on direct extraction. Thus it was shown above that the amount of growth substance extractable from coleoptile tips, 3–5 plant units, is produced hourly for at least 6 hours when the tips

are placed on agar. Since Boysen-Jensen has shown that growth substance diffuses out of root tips in a similar way if agar containing 10 per cent dextrose is used, experiments were carried out in which *Avena* root tips were placed on this agar. The roots were first well washed as before, and a number were lightly clamped together between

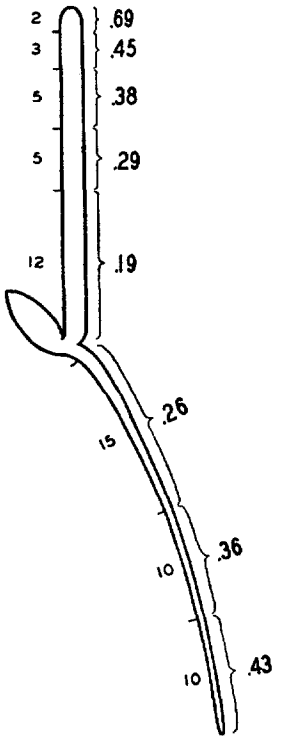

FIG. 2. Distribution of absolute growth substance concentrations in plant units per millimeter in the *Avena* coleoptile and root. The small figures indicate the lengths of sections used in millimeters.

pieces of cork, cut off at 10 mm. from the tip, and pressed gently upon the dextrose agar, contact being insured by means of a thin film of water. Diffusion took place in a moist chamber in the dark at 25°, and at the end of the experiment the root tips showed no drying out and appeared in good condition.

Confirming Boysen-Jensen's results, growth substance was readily

obtained in this way, the amount increasing with time of contact as shown in Table VI. However, in no case did the amount of growth substance exceed that obtainable by direct extraction, but on the contrary it appeared to approach that amount asymptotically. After 48 hours' diffusion a maximum was reached; after 72 hours the amount was somewhat less (Column 5, Table VI), possibly due to decomposition by microorganisms. Control agar blocks left in the same chamber for the same period did not develop any growth substance. Thus root tips behave in the opposite way from coleoptile tips and do not continue to produce growth substance when cut off.

This being the case, the only fair conclusion seems to be that growth substance is not produced in the root tip but merely accumulates there, being brought there by the polarity of its transport. This may explain why the presence of dextrose in the agar is necessary to draw it out, an osmotic gradient being thus set up which causes the growth substance to diffuse backwards. While in conflict with certain experiments in the literature, this view, based on direct growth substance determinations, seems to the author unavoidable. An alternative is that production of growth substance in the root tip, being dependent upon the supply of a precursor from the seed or plumule, ceases when this supply is cut off.

The results of the distribution experiments are summarized by Fig. 2, which expresses the growth substance in plant units per mm. in coleoptile and root. That the amounts in coleoptile base and root base are similar makes the above view reasonable.

SUMMARY

1. It is shown that when plant tissues are ground with water the growth substance contained therein is inactivated by the oxidizing enzymes.

2. A simple method of extraction is described which enables the quantitative determination of growth substance in such tissues.

3. The amount and distribution of growth substance in the *Avena* coleoptile is determined by this method, and it is shown that while the substance does not diffuse out from the lower parts of the coleoptile, it is nevertheless present in considerable amounts, the concentration decreasing steadily with the distance from the tip.

4. Growth substance is also present in considerable amounts in *Avena* roots, and here also its concentration decreases steadily with distance from the tip.

5. The amount of growth substance diffusing out of root tips into dextrose agar, even during long periods of time, is not greater than the amount obtainable by direct extraction. Actual production in the root tip therefore either does not take place at all, or else takes place under quite different conditions from the production in the tip of the coleoptile.

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